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The Influence of Various Soil Factors Upon Potato Scab caused by *Actinomyces scabies*

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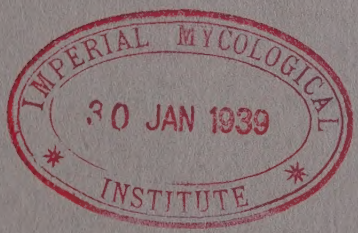
Department of Plant Pathology



LINCOLN, NEBRASKA
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SUMMARY

1. The effect of soil moisture, temperature, and aeration, and of the soil microflora as influenced by these factors, upon the occurrence of potato scab was studied under partially controlled conditions in the greenhouse. The influence of these factors upon the development of *A. scabies* in the soil preceding the infection period was emphasized in these experiments.

2. The severity of scab was found to be correlated with the amount of inoculum in the soil.

3. The amount of inoculum was in turn found to be affected by the degree of competition of other soil microörganisms.

4. Soil sterilization before inoculation resulted in the most severe scab. This effect could be greatly reduced by the addition of filtrates of unsterilized soil or of organic matter in the form of manure and by delaying inoculation until soil saprophytes had become established in the soil.

5. *A. praecox* added to the soil in amounts approximately equal to that of *A. scabies* did not reduce the severity of scab, as reported in the literature.

6. Sterilized soils inoculated with *A. scabies* and incubated at temperatures below 22° C. did not give rise to as much scab as those incubated at temperatures from 22° to 30°. (All were held at a temperature of 22° during the infection period.) Unsterilized soils did not show this effect of temperature upon the development of the pathogene.

7. The effect of soil moisture was variable. In the majority of the experiments the most scab occurred in the dry soils, but the occurrence in a number of experiments of severe deep scab in soils held at or near the saturation point did not indicate an effective control of the disease by high moisture, as reported in several publications.

8. The results of one experiment on soil types, while not conclusive, indicated that caution should be used in comparing the effects of soil moisture in different types of soil.

9. The largest amounts of scab occurred in soils that had been held at medium or high moisture contents for some months previous to planting. While results were not always consistent, there was no indication that high soil moisture had a depressing effect on the development of *A. scabies* in the soil, as determined by subsequent infection.

10. Counts of Actinomyces in soil dilution plates and on soil slides did not check with each other, nor did either show any consistent relationship to soil moisture or to scab. In general the percentage of Actinomyces was greatest in the drier soils, but the numbers were sometimes greater with high moisture, in contrast with the reported inhibition by high soil moistures.

11. A lack of soil aeration during the period preceding tuber formation had a greater effect than during the period of infection. In both cases scab was decreased.

12. The type of scab appeared to be determined by the host reaction, the environment, and the virulence of the culture. Common, deep, and raised scab often occurred on the same tuber in sterilized soil inoculated with a single pure culture of *A. scabies*. Superficial or russet scab occurred only in the unsterilized soils and may have been due to another strain or species of Actinomyces.

13. The development of an experimental technique which will permit the study of the pathogene in the soil immediately surrounding the susceptible plant tissues, as contrasted with the present gross methods, is essential for the determination of the effect of many of these soil factors upon the disease.

The Influence of Various Soil Factors Upon Potato Scab caused by *Actinomyces scabies*

R. W. GOSS

Scab, caused by *Actinomyces scabies* (Thaxt.) Güss., is a potato disease of major importance in western Nebraska. Seed treatments are effective in controlling seed-borne scab, but it has been shown that even when healthy or treated seed potatoes are used the disease may be very severe because of infection from the soil (7). Crop rotation will reduce the incidence of the disease, but the fact that potato scab may cause serious loss in soils never before planted to potatoes (7, 10) indicates that there are numerous factors other than the time interval between potato crops which affect the occurrence of the disease. A study of many of these factors has been undertaken in the field, greenhouse, and laboratory and the present publication deals with some of the results obtained under partially controlled conditions in the greenhouse.

High temperature, low moisture, alkalinity, abundant aeration, and the addition of barnyard manure are generally accepted, in the extensive literature on potato scab, as favoring the occurrence of the disease. On the basis of the published evidence, it should be possible to correlate the occurrence of the disease in the field with one or more of these factors. Unfortunately, however, the author has often found it difficult if not impossible to do this in many instances, as illustrated by the following examples.

During the past six years careful records have been made of the amount of scab occurring in all of the irrigated rotation plots at the Scotts Bluff Substation and of the unirrigated plots at the Box Butte Experiment Farm in western Nebraska. In addition, the inspection records of some 10,000 acres of potatoes being grown for certification have been examined each year. During this period there was one year, 1934, when extremely high temperatures and low rainfall prevailed. According to the literature this should have favored scab, but it was in this year that the least scab occurred. It was evident that further investigation of these factors was needed for a complete understanding of their influence on the disease.

It has been a common observation of many potato growers in western Nebraska that potato scab is always severe following heavy, packing rains when the field cannot be cultivated subsequently. Scab is also common in flooded areas at the lower ends of irrigated fields and in poorly drained portions of dry-land fields. This is the reverse of what one might expect from the work of Sanford (16) and Dippenaar (4) on soil moisture and Sanford's work (17) on the necessity of abundant aeration for the best development of the organism.

As regards the use of barnyard manure, the potato plots at the two substations previously mentioned, offer a striking contrast. The application of manure preceding potatoes has always resulted in a great increase of scab

in the irrigated Scotts Bluff rotation plots, while at the Box Butte farm, under dry-land conditions, no increase has been noted with similar applications and in many years the amount of scab has been materially less than in the unmanured plots.

No data are presented in this publication dealing with soil reaction. While all of the older publications point to the favorable effect of an alkaline soil on the disease it has recently been shown by several authors (5, 7, 18, 19) that highly alkaline soils may result in less scab than neutral or slightly acid soils.

It is evident from the above statements that further investigations are needed on the effects of environmental factors. There is a great lack of data on the effect of these factors on the organism in the soil, as most of the experimental evidence relates to pure cultures or is limited to the period of infection. It is impractical to change soil conditions greatly during the growth of the plant, with the one exception of soil moisture, but it is possible to modify the soil environment by rotations and cultural practices preceding the planting of potatoes. It was therefore considered desirable to place the emphasis in the following experiments upon conditions existing previous to infection, as this phase of the problem was in the greatest need of investigation.

EXPERIMENTAL METHODS

The experiments were conducted in a greenhouse held at a temperature of 20° to 23° C. In the first experiments (Tables 1 and 2) it was found that infected tubers occasionally were produced in the uninoculated controls grown in sterilized soil. This was eliminated in the later experiments by the use of individual saucers for each pot, careful watering, and the cleaning of floors and benches with water instead of by sweeping. In many experiments unsterilized soil was used and a small amount of slight scab usually occurred in these uninoculated soils. Unless otherwise specified, the following methods were used in all the experiments.

Soil.—The soil used was made up of three parts of bluegrass sod, one part sand, and one part stable manure. The bluegrass sod and manure were composted for a year or more. This compost was then run through a modified ensilage cutter before being placed in the soil bins. This treatment resulted in a fine, well mixed soil that did not require screening. Just before using, the compost was thoroughly mixed with river sand. Enough soil was prepared each fall to provide for all the experiments conducted during the winter. The soil thus prepared had a moisture-holding capacity of about 42 per cent as determined by the use of 25 cm. soil tubes.

When sterilized soil was required, the soil and containers were placed in a large steam sterilizer at 25 pounds pressure for 2½ to 3 hours.

Seed tubers.—The experiments were conducted with potatoes of the Bliss Triumph variety. Scab-free tubers were selected and treated with hot formaldehyde (1 to 120) for three minutes at 52° C., after which they were covered for one hour. When dry the tubers were cut into seed pieces weighing 40 grams each, which were held at a high humidity at room temperature for 24 hours to facilitate healing and were then treated with

ethylene chlorhydrin to break dormacy by dipping in a 3 per cent solution and placing in a closed container over night. They were then planted in sterilized sand. As soon as the sprouts and roots were well started, they were transplanted to the containers being used in the experiment and usually the soil was inoculated at this time.

Containers.—The plants were grown in seven-inch clay pots with individual saucers, except in some of the soil-moisture or aeration experiments when earthenware crocks or galvanized iron cans holding about 10 kilos of soil were used. When crocks or cans were used, the desired soil moisture was maintained by supplying water at the surface, which was covered with a half-inch layer of gravel, and also through glass tubes leading to an inverted two-inch clay pot filled with coarse gravel and placed in the center of the container. The amount of water needed was determined by frequent weighings. In some of the soil-moisture experiments the plants were grown in benches with the soil about eight inches deep instead of individual plant containers. Usually 24 plants were grown in each bench compartment, which was 30 square feet in size. This method had the advantage of providing a greater amount of soil per plant, and there was less variation in the soil moisture within each set of 25 plants than with clay pots or crocks, even with the daily weighings in the latter case.

Methods of recording scab.—Three types of scab will be referred to in this paper: **common,** i. e. definite, ruptured, corky lesions; **pit** or **deep,** i. e. lesions of about the same size as common scab but extending into the tuber about one-fourth inch and usually with the margins of such pits slightly raised; **superficial** or **russet** scab, i. e. diffuse, indefinite, brownish areas on the surface of the tuber. All these types of scab are pictured in Plate 1.

In all of the experiments the tubers produced were usually of a size fairly comparable with potatoes grown in the field. While it is often contended that the number of infections per tuber is the best method of recording scab, it was found to be too time-consuming. It also has the disadvantage of failing to differentiate between very small, inconspicuous scab spots and larger spots or deep scab pits. If the size and type of scab pustules are dependent to any extent upon tuber development, which is in turn influenced by soil environment, then the size and type of lesions become just as significant and as reliable an index as the number of infections per tuber. In field experiments and in the practical interpretation of the results of greenhouse tests we must consider the number or weight of tubers infected and proportion of surface infected. This was determined both on a weight and on a number basis. As the results were practically the same on each basis, usually only the number basis is used in the tables.

Dilution plates for quantitative determination of soil microörganisms.—Cultures were usually made from a composite of three to five soil samples. A 20-gram portion of each composite sample was added to 200 cc. of sterile tap water in a 500 cc. Erlenmeyer flask and shaken for five minutes. Dilutions were made to the desired concentration, one cc. of which was added to each petri dish. Seven petri-dish cultures of Waksman's albumen

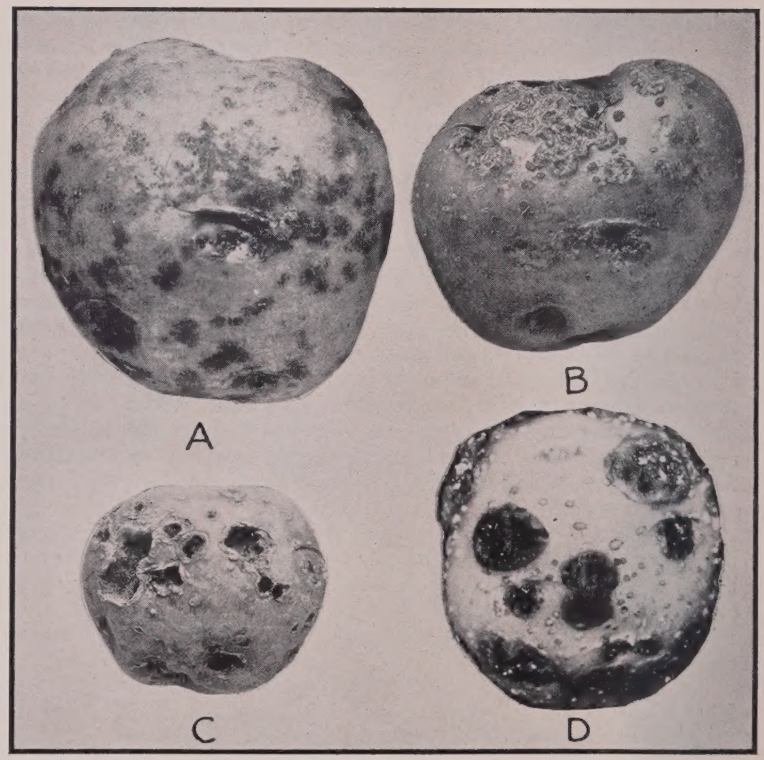

PLATE I

A. Russet or superficial scab on a Bliss Triumph potato grown in unsterilized soil (see Table 2).
B Common scab on a Bliss Triumph potato grown in soil inoculated with *A. scabies* N-23.
C. Deep scab on a Bliss Triumph potato grown in soil inoculated with *A. scabies* N-23.
D. Common scab on a potato grown in uninoculated sand cultures flooded with nutrient solutions several times daily. The proliferated lenticels show the effects of high moisture which failed to inhibit the development of scab (courtesy of H. O. Werner).

agar[1] were used for each determination of bacteria and Actinomyces, and an equal number of Jensen's asparaginate agar[2] were used for the determination of fungi. Counts of fungi were made after two to three days and of bacteria and Actinomyces after 10 to 14 days. Soil moisture determinations were made from other portions of each sample and all data presented in the tables are on a dry-weight basis.

[1] 1.0 gm. dextrose, 0.5 gm. K_2HPO_4, 0.2 gm. $MgSO_4$, trace $Fe_2(SO_4)_3$, 0.25 gm. egg albumen suspended in 5-10 cc. of water and neutralized to phenolphthalein with N/10 NaOH, 1,000 cc. water; pH 6.5.

[2] 20.0 gm. dextrose, 2.0 gm. asparagin, 1.0 gm. KH_2PO_4, 0.5 gm. $MgSO_4$, 0.5 gm. NaCl, 0.1 gm. $FeCl_3$, 30 gm. agar, 1,000 cc. water; pH 3.8-4.0.

Soil slide cultures.—In addition to the quantitative determinations of microörganisms by dilution plates, an attempt was made to check the results by use of Cholodny's slide technique (2). Clean glass slides were placed in the soil at a depth of 3 to 6 inches in a vertical position and in firm contact with the soil. These were left from 7 to 10 days and then fixed and stained by Conn's method (3) with rose bengal and examined under an oil immersion lens. About 200 fields on each of two or more slides were examined in each test.

Inoculum.—Cultures were prepared by inoculating the surface of potato dextrose agar in petri dishes with one cc. of a water suspension of the organism obtained from agar slants. This amount just covered the surface of the agar without undesirable excess water. After about two weeks' growth the surface of the petri-dish cultures was flooded with sterile water and the surface growth scraped and washed off, after which the suspension was filtered through fine muslin. The total inoculum for an entire experiment was made up in one container and 200 cc. of this per plant was used to inoculate the soil.

The concentration of the inoculum was determined by the following preliminary test, the results of which are presented in Table 1. Sterilized soil was inoculated, at the time tubers started to develop, with 240 cc. of inoculum per pot in five different concentrations. The undiluted inoculum of *A. scabies* N-23 contained three petri-dish cultures to each 240 cc. of water, which was the amount of inoculum used in each pot of Set 1. For each of the next four sets, 0.1, 0.01, 0.001, and 0.0001 dilutions of this inoculum were used; and an additional set was left uninoculated as a control. Each set contained 18 plants, except the control, which had only 10.

TABLE 1.—*Influence of the amount of inoculum upon the production of scab.*

Set No.	Amount of inoculum	No. of tubers	Classes of tubers, determined by percentage of surface scabby				
			0	0-2	2-25	25-75	75-100
		No.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. Ct.*
1	Undiluted [1]	76	9	12	50	26	3
2	1-10 dilution	72	14	37	46	3	0
3	1-100 dilution	63	33	52	14	0	0
4	1-1,000 dilution	64	56	41	3	0	0
5	1-10,000 dilution	73	78	22	0	0	0
6	Check	38	92	8	0	0	0

[1] The undiluted inoculum was at the rate of three petri-dish cultures, two weeks old, in 240 cc. of water per plant.

The data presented in Table 1 show that the percentage of tubers infected and the scabby area per tuber were both directly correlated with the concentration of the inoculum. In addition, pit scab occurred only in Sets 1 and 2, which had the greatest concentrations.

It is evident that the amount and severity of scab in this test was directly dependent upon the amount of *A. scabies* in the soil, and that good results could be secured in sterilized soil by the use of one to three petri-dish cultures per plant. Accordingly, in all of the following experi-

ments the inoculum was used at the rate of one and one-half petri-dish cultures in 200 cc. of water for each plant.

The inoculum was thus of only approximately the same strength in different experiments and with different cultures of Actinomyces, but it was uniform for the individual plants in each experiment. The inoculum prepared in this way will be referred to in this paper as a spore suspension, although this term is not strictly correct.

In all experiments, unless otherwise noted, the inoculum was prepared from cultures of *A. scabies* N-23. This strain was isolated from lesions of the pit scab type on Bliss Triumph potatoes produced in western Nebraska in 1928.

INFLUENCE OF SOIL MICROFLORA AND OTHER FACTORS

In many of the experiments reported in the literature, the soil has been sterilized before inoculation. It is obvious that sterilization changes the physical and chemical nature and upsets the biologic equilibrium of the soil. The effect of any one limiting factor may thus be greatly modified and results obtained in this way are not necessarily a true index of what would occur under field conditions. This has been recognized by a few workers who conducted their tests with unsterilized soils. Many of the experiments reported in this bulletin were conducted with unsterilized soil and were duplicated with sterilized soil for comparison with published data of a similar nature.

Preliminary studies were made of the effect of soil sterilization and to throw some light on the question whether any differences in the occurrence of scab were due to chemical or physical changes in the soil or to the lack of competing organisms. This was tested by adding to sterilized soil an extract of unsterilized soil, filtered through muslin. At the same time the effect of the time of inoculation was determined by adding the inoculum either at the time of planting or at the beginning of tuber formation.[3] The data regarding the effect of these treatments are presented in Table 2.

Among the different soils with similar inoculations, scab infection was greatest in the sterilized soil of Set 1, where it was very severe, while Set 2, to which a nonsterile filtrate was added, produced only slightly more scab than the unsterilized Set 3.

These results offer presumptive evidence that the greater amount of scab developing in the sterilized soil was due to a lack of competing soil microörganisms. This was further indicated by the fact that while there were no significant differences in Sets 2 and 3 due to time of inoculation, there was less severe scab in the sterilized Set 1 with the late inoculation made at the time of tuber formation (1 B), possibly because of the competition of many organisms that had become established in the soil after sterilization and before the inoculum was added.

[3] In this and in many of the following experiments and in the tabulated data, the beginning of tuber formation or "time of tuber set" has been used in an arbitrary manner to divide the growth period of the plant into two general phases. It was determined by examining the stolons of a number of plants in each set and whenever tuber initiation was evidenced the time was considered as that of "tuber set," although it is recognized that tubers were initiated on many plants after this time and on a few plants before this time.

TABLE 2.—*Effect of soil sterilization and time of inoculation upon scab development and upon the numbers of bacteria and Actinomyces.*

Set No.	Inoculation	Number of plants	Weight of tubers	Number of tubers	Classes of tubers, determined by percentage of surface scabby [1]					
					0	0-2	2-25	25-75	75-100	Russet
		No.	*Grams*	*No.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
				STERILIZED SOIL						
1A	At planting.....	18	3,165	88	0	0	10	24	66	0
1B	At tuber formation.	20	3,895	97	0	7	30	36	27	0
1C	Uninoculated	10	....	35	66	14	17	3	0	0
				STERILIZED SOIL PLUS FILTRATE OF UNSTERILIZED SOIL [2]						
2A	At planting.....	20	4,270	67	0	15	43	21	3	18
2B	At tuber formation.	18	3,860	68	0	13	48	25	2	12
2C	Uninoculated	10	2,330	43	0	7	5	0	0	88
				UNSTERILIZED SOIL						
3A	At planting.....	20	3,880	61	0	26	44	15	0	15
3B	At tuber formation.	19	3,590	56	0	14	34	20	0	32
3C	Uninoculated	10	1,960	32	0	6	16	0	0	78

Set No.	Inoculation	Bacteria per gram (dry weight)		Actinomyces per gram (dry weight)		Time of sampling	
						After sterilization	After inoculation
		Millions	*P.ct*	*Millions*	*P.ct.*	*Days*	*Days*
		STERILIZED SOIL					
1A	At planting.....	176.6	76	55.7	24	82	78
1B	At tuber formation.....	250.8	91	25.2	9	82	35
1C	Uninoculated	292.8	97	8.5	3	82	..
		STERILIZED SOIL PLUS FILTRATE OF UNSTERILIZED SOIL [2]					
2A	At planting	....	..	....	..	..	..
2B	At tuber formation.....	....	..	....	..	..	..
2C	Uninoculated	80.2	90	9.1	10	82	..
		UNSTERILIZED SOIL					
3A	At planting	....	..	....	..	..	..
3B	At tuber formation.....	....	..	....	..	..	..
3C	Uninoculated	35.3	78	9.7	22	..	.. [3]

[1] Percentages based on number of tubers.
[2] Filtrate obtained from about 1 pound of soil by adding water and allowing it to stand overnight and then filtering through muslin. 300 cc. of filtrate was added to each plant.
[3] Average numbers of three dates of sampling.

Russet or superficial scab developed only in the unsterilized soil or when a nonsterile filtrate was added, and in these soils it was most common in the uninoculated checks. It may well be that this type of scab was caused by a different species of Actinomyces that was present in the original soil. The other types of scab varied from common scab to deep pits. There was a small amount of slight infection in the uninoculated controls.

The numbers of Actinomyces in the soil, as determined by the dilution plate method, checked very well with the incidence of scab in the various sets, as shown in Table 2. The largest number of Actinomyces 82 days after sterilization occurred in Set 1A, which had been inoculated for the longest period of time and in which the inoculum had apparently become well established before the soil became infested with competing organisms. The later inoculation in Set 1B had the next highest number of Actinomyces and the least number occurred in Set 1C, which was not inoculated.

The amount of scab produced, as recorded in Table 2, appeared to be directly correlated with the total number rather than with the percentage of Actinomyces. A comparison of the uninoculated sets shows that the number of Actinomyces was least in the uninoculated controls. The effect of sterilization on the uninoculated soils was very noticeable in the high numbers of bacteria in Set 1C, while the addition of the nonsterile filtrate in Set 2C resulted in a much lower number. Ordinarily the numbers of bacteria increase rapidly in a sterilized soil but eventually decrease to the approximate number present before sterilization. In Set 2C the soil was immediately treated with a filtrate of unsterilized soil and the maximum number of organisms had probably occurred previous to the time of sampling. This process was much slower in 1C, which depended upon chance contamination.

TABLE 3.—*The effect of the competition of soil microöganisms upon the occurrence of scab in inoculated soils.*

Set No.	Treatment of soil [1]	Number of plants	Number of tubers	Classes of tubers, determined by percentage of surface scabby			
				0	0-2	2-25	25-100
		No.	*No.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
	STERILIZED SOIL, INOCULATED WITH *A. scabies*						
1	No treatment	58	249	2	6	34	58
2	Filtrate of unsterilized soil.	34	176	8	17	27	48
3	Filtrate of sterilized manure	40	205	7	8	36	49
4	Sterilized manure	20	102	12	13	49	27
5	Filtrate of unsterilized manure	39	215	20	22	34	24
6	Unsterilized manure	20	78	54	17	23	6
7	Penicillium sp.	30	160	1	4	35	60
8	Bacteria	30	160	9	9	38	44
9	Actinomyces 91 (*saprophytic*)	20	103	7	7	48	39
10	Mixture of treatments of 7, 8, 9	19	94	2	2	52	43
	STERILIZED SOIL, NOT INOCULATED						
11	No treatment	20	85	100	0	0	0
	UNSTERILIZED SOIL, INOCULATED WITH *A. scabies*						
12	No treatment	50	211	17	25	39	19

[1] All treatments were made at the time of inoculation. All inoculations were made at the rate of 200 cc. of inoculum per pot, equal to 1½ petri-dish cultures. Saprophytic organisms were added at the same rate. The filtrates were obtained by soaking 4 parts of soil or manure in 6 parts of water over night, filtering through cheesecloth, and adding 200 cc. per pot. The manure in Sets 4 and 6 was added at the rate or 200 grams per pot.

Additional tests were made both of the effect of the addition of sterilized and nonsterilized organic matter and of the filtrates of the same to sterilized inoculated soil. Three types of organisms isolated from the unsterilized soil were cultivated and added to the soil in amounts approximately equal to that of *A. scabies* in an effort to determine whether either of these particular groups were effective competitors of the scab organisms in the soil. These tests were made during different years in the greenhouse but as the results were of the same general nature they have been compiled into one composite table (Table 3).

As in the previous experiment, there was much less scab in the unsterilized soil (Set 12) than in the sterilized soil (Set 1) similarly inoculated,

the difference probably being due to the lack of competition of microörganisms in the sterilized soil. The addition of a filtrate from unsterilized soil (Set 2) did not have as great an effect as in the preceding experiment, although there was a decrease in the severity of scab that is not clearly indicated in Table 3. Only one-third of the severe scab in Set 2 was of the very severe type, as contrasted with Set 1 in which one-half of that listed in the table as severe was of this very severe type (more than three-fourths of the surface of the tuber scabby). The addition of a filtrate of sterilized manure (Set 3) had very little effect, but the addition of organic matter in the form of sterilized manure (Set 4) decreased the disease considerably. This decrease could not have been due to organisms added, nor to organic acids or other substances also present in the filtrate added to Set 3, which did not reduce scab. Possibly this added organic matter so changed the soil

TABLE 4.—*Effect of competition of A. praecox with A. scabies upon scab.*

Treatment	Number of plants	Total grams or number	Classes of tuber, determined by percentage of surface scabby				
			0	0-2	2-25	25-75	75-100
	No.	*Grams*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
		ON WEIGHT BASIS					
Check	15	750	100.0	0	0	0	0
A. praecox 3374	15	1.210	100.0	0	0	0	0
A. scabies N-23	30	3.180	25.1	39.3	18.9	14.2	2.5
A. scabies + *A. praecox*	30	3.375	26.7	35.6	16.3	19.3	2.2
		ON NUMBER BASIS					
Check	15	46	100.0	0	0	0	0
A. praecox	15	50	100.0	0	0	0	0
A. scabies	30	93	24.7	43.0	18.3	10.8	3.2
A. scabies + *A. praecox*	30	105	28.6	41.0	16.2	12.4	1.9

texture that it acted indirectly upon the development of scab through changes in aeration and moisture-holding capacity of the soil. It is more probable, however, that this additional favorable substratum resulted in a more rapid increase in the number of microörganisms resulting from chance contamination following sterilization and that the number of Actinomyces were inhibited by these competing organisms. This is further borne out by the fact that the filtrate from unsterilized manure (Set 5), containing many microörganisms, decreased the amount of scab more than the sterilized organic material in Set 4 and the addition of unsterilized manure in Set 6 containing both the microörganisms and the favorable organic substratum resulted in less scab than any other treatment under greenhouse conditions.

The addition of cultures of saprophytic forms of Penicillium, bacteria, and Actinomyces, and mixtures of all three in amounts equal to that of the inoculum, failed to have any appreciable effect upon the occurrence of scab.

The tests with *A. praecox* Millard and Burr [4] were made in 1935-36 under conditions where the amount of scab produced by *A. scabies* was not as great as in the previous tests and the results are presented in Table 4.

[4] Culture obtained through the courtesy of Dr. S. Waksman.

These results fail to reveal any antibiotic or inhibitory action of *A. praecox* on *A. scabies,* as judged by the amount of scab produced on tubers with approximately equal amounts of the two organisms added to sterilized soil. This is not in agreement with results of Millard and Burr (15).

The preceding experiments were made with a composted soil of high fertility and the results would not be strictly applicable to field soils. Similar experiments therefore were planned with unsterilized field soils obtained from the upper 6 to 8 inches of potato fields in western Nebraska. These soils were mostly very fine, sandy loams or silt loams in the Rosebud series, the pH of which varied from 6.8 to 8.1. The tests were made during three successive winters and the results are all brought together in Table 5.

Some of the results presented in Table 5 are difficult to interpret in comparison with those obtained in the preceding experiments with composted soil. The only significant reduction in scab as compared with the

TABLE 5.—*The effect of various treatments of field soils upon the occurrence of scab in the greenhouse.*

Set No.	Soil treatment [1]	Number of tubers	Classes of tubers, determined by percentage of surface scabby			
			0	0-2	2-25	25-100
		No.	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
1	No treatment	77	13	14	35	38
2	Sand added	68	33	5	27	35
3	Manure added	99	39	20	20	21
4	Sterilized manure	147	55	24	16	5
5	Filtrate of manure (200 cc. per pot)	30	12	4	30	54
6	Sand and filtrate of manure	24	0	0	29	71
7	Green manure (alfalfa or grass cuttings)	72	14	21	30	35

[1] The sand and manure were each added at the rate of one part to two parts of soil by volume. The alfalfa or grass cuttings were added at the rate of 100 grams green weight per pot, about six weeks before planting. The filtrate of manure was obtained as in the preceding tests.

controls (Set 1) occurred in Sets 3 and 4 with the addition of manure. The effect of added organic matter was similar to the results presented in Table 3. It is difficult to understand, however, why the sterilized manure in Set 4 resulted in less scab than the unsterilized manure in Set 3, or why the addition of a filtrate of manure, either alone (Set 5) or with sand (Set 6), failed to decrease the amount of infection. In fact the percentage of infection was even higher in the latter two sets than with untreated soil (Set 1.) This may not be a significant difference, however, as the numbers of tubers in these two sets were rather small. These field soils were much lower in organic matter than the composted soils of the previous experiments, so that the addition of organisms in a filtrate would probably have less effect than in previous tests. The addition of green manure in the form of alfalfa had no appreciable effect upon the amount of scab.

In addition to the test reported in Table 5, three lots of field soil were held for six weeks before planting as follows: (1) frozen, (2) air dry in sunlight, and (3) moist. The total amount of scab resulting was about the same in each case and did not appreciably differ from the untreated soil, except that the soil that had been dried in a thin layer in sunlight produced less of the severe type of scab.

EFFECT OF TEMPERATURE OF THE SOIL PRECEDING TUBER FORMATION

Many of the experiments reported in the literature on the effect of soil temperature upon the development of scab, have been based upon soil held at a constant temperature throughout the experiment. The effect of sterilizing the soil and the effect of soil temperature upon the pathogene in the soil before tuberization usually have not been considered. The following experiment was designed to throw some light upon these two points.

The soil was divided into two parts, one part being sterilized; and both portions were then inoculated with *A. scabies* N-23, applied to the soil as

TABLE 6.—*The effect of the soil temperature preceding tuber formation upon the development of scab at 22° C.*[1]

Soil temperature preceding tuber formation	Number of tubers	Classes of tubers, determined by percentage of surface scabby				
		0	0-2	2-25	25-75	75-100
Deg. C.	*Number*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
SOIL STERILIZED BEFORE INOCULATION						
6	13	0	8	38	31	23
10	12	0	0	8	42	50
14	16	0	0	0	31	69
18	8	0	0	0	37	63
22	10	0	0	0	0	100
26	11	0	0	0	0	100
30	10	0	0	0	10	90
SOIL NOT STERILIZED BEFORE INOCULATION						
6	13	0	15	31	31	23
10	8	0	0	0	75	25
14	11	0	0	46	46	9
18	8	0	0	13	63	25
22	11	0	9	9	73	9
26	12	0	8	8	33	50
30	10	0	0	10	60	30

[1] Soils at 6° and 10° C. were held at those temperatures for 70 days. All others were held at the specified temperatures for 89 days before transferring to 22° C. for tuberization, 33 days after planting.

it was being thoroughly mixed before potting. The moisture content of the soil at the time it was inoculated was brought up to about 20 per cent moisture dry weight, which was equal to 50 per cent of the moisture-holding capacity, and it was held at that point by daily watering based on weight.

The experiment was divided into seven sets of eight plants each, four in sterilized and four in unsterilized soil. These sets were placed in soil-temperature tanks held at the following temperatures: 6°, 10°, 14°, 18°, 22°, 26°, and 30° C. After inoculation the soils were held at these temperatures for 55 days before the seed pieces, previously sprouted in sterile sand, were transplanted.

The plants at 6° and 10° C. developed so slowly that they were transferred to a temperature of 22° C. 15 days after the seed pieces were transplanted, while all the other sets were transferred 10 days later, when tuber

development was just starting at the optimum temperature. Thus the sets at 6° and 10° were held at those temperatures for a total of 70 days and the other sets were held at the desired temperatures for 89 days before they were all placed at the same room temperature of 22° C.

The effect of the soil temperature preceding tuber formation upon the development of scab is shown in Table 6. No healthy tubers were produced in this experiment. The greatest amount of scab occurred with soil sterilized before inoculation and held at 22° and 26° for 89 days. Slightly less scab occurred in sterilized soil held at 30° and smaller amounts with the lower temperatures, particularly at 6° and 10°. In the soil that was not sterilized there were no consistent differences in the amount of scab in soils held at the different temperatures.

TABLE 7.—*Bacteria and Actinomyces per gram of soil (dry weight) in sterilized soil, held at various temperatures, after inoculation with Actinomyces scabies.*

Soil temperature	21 days after inoculation			54 days after inoculation			89 days after inoculation [1]		
	Bacteria	Actinomyces		Bacteria	Actinomyces		Bacteria	Actinomyces	
Deg. C.	*Millions*	*Millions*	*P.ct.*	*Millions*	*Millions*	*P.ct.*	*Millions*	*Millions*	*P.ct.*
6 [1]	3.7	0.1	2.6	66.4	0.1	0.2	171.8	14.3	7.7
10 [1]	8.5	0.1	1.2	65.4	2.0	3.0	149.5	17.5	10.5
14	27.9	6.6	19.1	81.0	31.3	27.9	89.9	78.2	46.5
18	32.1	19.1	37.3	88.7	49.0	35.6	59.0	87.0	59.6
22	35.9	29.8	45.4	126.0	61.0	32.6	100.9	80.6	44.4
26	94.0	49.5	34.5	88.3	48.8	35.6	85.1	48.7	36.4
30	142.0	58.0	29.0	...	...	...	50.9	42.8	45.7

[1] The sets at 6° and 10° C. were changed to 22° C. 70 days after inoculation, or 19 days before the third sampling. For data on scab, see Table 6.

Soil samples were collected from the sterilized soils 21, 54, and 89 days after inoculation and the number of bacteria and Actinomyces were determined by dilution plate cultures (Table 7). The number and percentage of Actinomyces could be correlated with the amount of scab produced in the sterilized soil as shown in Table 6. Fewer Actinomyces occurred in the soils held at 6° and 10° C. than at the higher temperatures and these were the same soils that had the least scab. At the highest temperatures, 26° and 30°, the greatest total number of bacteria and Actinomyces was reached within 21 days, while the highest number at 22° was reached at the next sampling and at the lower temperatures at the third sampling. The number and percentage of Actinomyces remained relatively constant at 26° C. The percentage of Actinomyces did not change greatly at 22°, although the number increased at each sampling. At 10° and 14° both the number and percentage tended to increase with increased time intervals up to the 89-day period. The great increase in both bacteria and Actinomyces at the third sampling was undoubtedly due to the fact that these soils were changed to 22° C. 19 days before the final sampling.

In the soils that were not sterilized before inoculation the average numbers of bacteria and Actinomyces were much less than with the steri-

lized soils, averaging 5, 11, and 15 million, respectively, on the three successive dates of sampling without any consistent differences in the total number of Actinomyces as a result of exposure to the different temperatures for different periods of time. There was, however, a decrease in the percentage of Actinomyces at the lower temperatures, being 18 per cent at 6° and 21 per cent at 10° C., while at the other temperatures the percentages were between 24 and 29. There was no increase in the percentages with successive sampling as in the sterilized soil.

It is clear that the results obtained following sterilization are not necessarily an indication of what would occur under field conditions. The effects of soil temperatures in the sterilized soil as presented here do, however, explain the differences in the magnitude of the effect of soil temperatures when held constant throughout the life of the plant, as in the results presented by Dippenaar (4), who, working with unsterilized soil, did not find as great temperature effects as Jones, McKinney, and Fellows (9), who worked with sterilized soil.

The greater infection in sterilized soil may be partially due to the manner of growth of Actinomyces. In this experiment two counts of Actinomyces colonies were made, one at the end of 4 to 5 days, the other after 10 to 14 days. McLennan (13) has shown that counts taken in this way with fungi can be used to differentiate spores and mycelium. While no work of this nature has been reported with Actinomyces, it is interesting to note that if they behave similarly to the fungi we may assume that the colonies appearing at the first reading are mycelial fragments and those at the second reading are both spores and mycelium. On this basis counts made on these soils indicate that when the cultures were made 54 days after inoculation, 84 per cent of the colonies in sterilized soil and 66 per cent in the nonsterilized soil were from mycelium. There is a great need for investigations dealing with the effect of environmental factors on the morphological development of soil-inhabiting pathogenes of this type before the effect of the various factors can be correctly interpreted.

The single experiment here reported indicates that temperature may have an effect on the scab-producing power of the soil but that the effect is very slight in unsterilized as compared with sterilized soils and more extensive experiments than were here possible are needed before definite conclusions can be drawn.

THE RELATION OF SOIL MOISTURE TO THE DISEASE

Sanford (16, 17) and Martin (12) concluded from their experimental evidence that high soil moisture is a limiting factor in the development of potato scab. Sanford (17) also concluded, from his study of soil moisture in relation to the critical period for infection, that high soil moisture during the early stages of tuber development would prevent scab even though the soil became dry later in the season.

Dippenaar (4) not only agrees regarding soil moisture as a limiting factor but further concludes that even after the tubers have become infected they will recover and slough off the diseased tissue when the moisture

content is raised, if they are still actively growing. He does not agree with Sanford (16) that the influence of soil moisture is largely limited to the early stages of tuber development, although he does consider that the maximum influence is exerted during the first four weeks after the tubers are set. Dippenaar's conclusions are largely based upon an experiment in which the soil moisture content was changed at intervals beginning 30 days after emergence, and he assumes on the basis of Fellows' work (6) that all of the tubers of an individual plant are set at about the same time. It has been shown, however, by Werner (20) and others, that plants in a highly vegetative state may differentiate tubers over a considerable period of time and that the time, duration, and extent of tuber setting can be altered considerably by environmental factors.

In contrast with the above, some of the older papers on this disease report an increased amount of scab with increased moisture. Beckwith (1) found that when scabby seed was irrigated there was 57 per cent of scab, as contrasted with 32 per cent for the unirrigated. On manured soils he found an even greater difference, 71 and 30 per cent respectively. It has been previously mentioned that field observations in western Nebraska have revealed that potato scab is usually more severe in years of high rainfall and of little importance in drouth years. In 1933 the certification records showed an average of 11.2 per cent of scab culls in potatoes from over 300 fields in western Nebraska when the effective rainfall [5] for the period of tuber development, August and September, was 4.43 inches. In contrast with this only 1.4 per cent of scab of a similar degree of severity was present in 1934, when the effective rainfall for the same period was only 1.7 inches. These results conform with the general experience of farmers in that area over a period of years. It is also a matter of record that scab is just as great a problem under irrigation as in the dry-land areas. In a preliminary test conducted at the Scotts Bluff Substation it was found that withholding the last irrigation (August 17) did not affect the amount of scab as compared with those receiving an additional irrigation. The author has seen severely scabbed potatoes produced in sand cultures where the tubers were only one to two inches above the water level and in others where the sand was flooded with nutrient solution several times a day. The moisture content was high enough to cause proliferation of the lenticels on these scabby potatoes (Plate 1).

The above statements have dealt primarily with the disease, but there remains the question of just how the soil moisture exerts its influence. Dippenaar (4), employing the Cholodny slide technique, came to the conclusion that high soil moisture favored the development of bacteria which were antagonistic to the scab organism and that spore germination and growth of *A. scabies* were inhibited under these conditions. Sanford (17), using the dilution plate method, decided that the development of the causal organism in the soil is retarded by either very low or high moisture contents, although he found the disease in maximum amounts in very dry soil. Supplementary but indirect evidence of the effect of soil moisture may be

[5] Effective rainfall is here considered at 0.25 inch or more in 48 hours.

found in the numerous statements in soil bacteriological literature to the effect that the Actinomycetes in general are favored, in comparison with the bacteria, by low soil moistures.

On the other hand, Lutman, Livingston, and Schmidt [11], using the dilution plate method of studying bacteria and Actinomyces in relation to the soil moisture of field soils during different seasons of the year, came to a different conclusion. They found that the Actinomyces counts roughly paralleled those of the total number of organisms, which were highest in winter with high soil moistures and lowest in summer with low soil moistures. They did not find any evidence of antagonism between bacteria and Actinomyces. The author [7] found by plating out samples of some 100 field soils, varying in moisture content from 8 to 32 per cent, that the percentage of Actinomyces was highest in the medium moisture group and lowest in either the extremely dry or wet soils, although the total number of organisms was highest in the high moisture samples.

One phase of the soil moisture problem that has received relatively little attention is that of the effect of different types of soils. It is possible that what might be considered low rainfall in one section of the country would be a high rainfall in another section, as regards both actual precipitation and moisture-holding capacity of the soil. Inasmuch as the soil microörganisms live primarily in the colloidal film surrounding the soil particles, it is evident that in experiments with controlled water supply the lower the colloidal content of the soil the smaller will be the amount of water necessary for microbiological activities. Excess moisture in a soil would be expected to inhibit the growth of aerobic organisms. It was therefore thought possible that the amount of water present in proportion to the water-holding capacity of the soil might have a greater influence on the disease than the actual water present on a dry-weight basis.

There have been practically no studies dealing with the effect of soil moisture and other factors upon the development of the pathogene in the soil preceding the infection period. In most of the studies constant soil moistures have been employed or the moisture content has been varied, as in Dippenaar's work [4], during the period of tuber development. The emphasis throughout has been placed upon the conditions essential for infection. Because of the many contradictory statements in the literature and the author's inability to correlate the occurrence of scab in the field with much of the published data, it was felt that other factors must be involved and that further work was needed before an adequate explanation of the relation of soil moisture to the disease could be found. The following experiments were planned to determine three points: first, the effect of soil moisture during the period of infection; second, the influence of soil moisture preceding the period of tuber formation upon the development of the causal organism and as a result the scab-producing power of the soil; and third, the effect of soil moisture in different types of soils varying in their moisture-holding capacity.

Soil Moisture Experiment 1.—The first preliminary experiment employed the soils previously used in the soil temperature experiments. These

were originally in two sets, sterilized and unsterilized, and the soils of each set were thoroughly mixed and then divided into high and low moisture sets, one being held near the minimum for growth and the other above the optimum. The plants were grown in seven-inch clay pots and the relative moisture contents were maintained approximately without weighing.

TABLE 8.—*The effect of soil sterilization and moisture content upon scab folowing inoculation with A. scabies (Soil Moisture Experiment 1).*

Set No.	Soil treatment	Number of tubers	Weight of tubers	Classes of tubers determined by percentage of surface scabby [1]				
				0	0-2	2-25	25-75	75-100
		No.	*Grams*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
1	Sterilized—high moisture	33	1,460	1	4	35	34	26
2	Sterilized—low moisture	41	900	0	0	3	0	97
3	Not sterilized—high moisture..	36	1,795	3	12	84	1	0
4	Not sterilized—low moisture...	38	1,210	0	0	21	15	64

[1] Data based upon tuber weights.

It is evident from Table 8 that low soil moisture greatly favored the development of scab. Even with the high soil moisture severe scab occurred in the soil that had previously been sterilized. The effect of soil moisture, however, was greater than that of sterilization.

Soil Moisture Experiment 2.—In this experiment the plants were grown in three-gallon earthenware jars and moisture requirements were determined by frequent weighings. Sterilized and unsterilized soils were used, and each was divided into moisture sets of 20 plants each held at 30, 50, and 70 per cent of the moisture-holding capacity, which was 58.7 per cent dry weight as determined by the use of one-centimeter cups. The soil was inoculated with *A. scabies* N-23 at the rate of two petri-dish cultures per plant at the time the sprouted seed pieces were transplanted.

The plants grown in the sterilized soil, which were larger and more vigorous than those in unsterilized soil, developed an abnormal condition about five weeks after the start of the experiment. Many of the plants were wilting and a number started to develop small tubers at the surface of the ground, similar to those often found in the field as a result of the death of the underground stem due to Rhitzoctonia or other causes. Later examination showed that the underground stems of these plants were severely infected with *A. scabies*. This infection sometimes involved all tissues of the stem and resulted in a soft rot. Infection of the roots, particularly at the base, was also noted. Cultures made from such stems and roots as well as microscopic examination of sections always revealed the presence of Actinomyces. This wilting, caused by stem and root infection, occurred only in the sterilized soils and was more severe at the low moisture contents where six plants wilted as compared with four at the medium moisture and three at the high moisture content. At the close of the experiment it was found that 60 per cent of the stems were infected in the unsterilized soil but that infection was not severe enough to cause wilt, while 91 per cent of infection had occurred in the sterilized soil.

It was evident from these results that the inoculum applied was probably excessive and that the lack of competition, particularly in the sterilized soils, allowed the pathogene to develop profusely and cause a severe infection of stems and roots. While no reports are available of such severe attacks in the field, it is evident that the organisms can produce a stem rot under some conditions at least. Later findings have shown that Actinomyces are more commonly present in stem and root lesions and in root hairs of potato plants in the field than has previously been reported. L. R. Jones, McKinney, and Fellows (9) report similar infection but of a less severe type in their soil-temperature experiments and A. Powell Jones (8) describes infection of roots and etiolated shoots in his histological study of the disease.

TABLE 9.—*The effect of soil moisture upon scab development (Soil Moisture Experiment 2).*

Soil moisture as percentage of moisture-holding capacity	Number of tubers	Classes of tubers, determined by percentage of surface scabbed				
		0	0-2	2-25	25-75	75-100
P.ct.	*No.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
		STERILIZED SOIL				
30	63	9	8	16	13	54
50	80	5	5	14	31	45
70	102	2	14	23	36	25
		UNSTERILIZED SOIL				
30	47	2	25	32	26	15
50	69	45	13	26	12	4
70	103	29	26	28	14	3
Analysis of the low-moisture sets on the basis of soil surrounding each individual tuber [1]						
Tubers in dry soil	28	25	32	32	11	0
One-half of tuber in dry soil	34	0	18	35	35	12
Tubers in wet soil	43	0	4	8	10	77

[1] See discussion of data.

Scab was much more severe in the sterilized than in the unsterilized soils and it was likewise more severe with low moisture (Table 9). The effect of soil moisture was partially obscured by the severity of infection in all the sets with sterilized soil.

A more detailed examination of the tubers in the low-moisture sets revealed an unexpected condition. The soil in these was very dry at the start and the small amount of water added at each weighing did not become equally distributed through the soil. As a result the soil immediately surrounding the inverted clay pot to which the water was added had a much higher moisture content than that in the rest of the container. It was noted that the tubers tended to form at this point and many tubers therefore were formed in such a position that part of the tuber was in contact with the moist soil and the rest of the tuber surrounded by dry soil. By careful examination of the position of each tuber at digging, it was found that scab had developed only on that portion of the tuber in contact with the moist soil. In Table 9 is recorded the amount of scab in relation to the position of the tuber as regards soil moisture. Both the sterilized and un-

TABLE 10.—*The effect of the soil moisture (in relation to the moisture-holding capacity) and of previous sterilization upon the occurrence of scab and the numbers of bacteria, Actinomyces, and fungi (Soil Moisture Experiment 3).*

Water (dry weight)	Ratio water to moisture-holding capacity	Tubers	Classes of tubers, determined by percentage of surface scabby					Microörganisms per gram of soil (dry weight) [1]											
								71 days after inoculation						95 days after inoculation					
			0	0-2	2-25	25-75	75-100	Bacteria		Actinomyces		Fungi		Bacteria		Actinomyces		Fungi	
P.ct.	*P.ct.*	*No.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*Mill.*	*P.ct.*	*Mill.*	*P.ct.*	*Thous.*	*P.ct.*	*Mill.*	*P.ct*	*Mill.*	*P.ct.*	*Thous.*	*P.ct*
COMPOST SOIL—STERILIZED																			
20.9	26	18	50	17	33	0	0	339.0	66.2	173.0	33.8	....	..	484.8	61.0	309.7	39.0	....	..
36.2	45	13	8	23	46	23	0	106.0	92.3	8.8	7.7	....	..	96.4	66.3	49.0	33.7	....	..
60.2	75	33	9	21	27	18	25[2]	53.9	86.3	3.6	13.7	....	..	87.1	96.2	3.4	3.8	....	..
COMPOST SOIL PLUS SAND STERILIZED																			
11.1	26	22	4	23	27	18	27	226.0	66.4	115.0	33.6	....	..	256.0	49.5	261.0	50.5	....	..
19.2	45	25	4	8	28	32	28	111.0	95.4	5.3	4.6	....	..	58.0	87.8	8.1	12.2	....	..
31.9	75	26	4	4	69	12	12	54.1	97.4	1.4	2.6	....	..	83.9	98.2	1.5	1.8	....	..
COMPOST SOIL—NOT STERILIZED																			
20.9	26	20	15	30	50	5	0	19.0	68.6	8.5	30.8	193.7	0.7	28.3	66.6	14.0	33.0	184.0	0.4
36.2	45	14	21	7	57	14	0	20.5	68.3	9.3	31.2	163.0	0.5	24.0	68.0	11.1	31.4	209.0	0.6
60.2	75	27	0	56	41	4	0	22.8	71.0	9.1	28.4	235.0	0.7	27.5	68.8	12.2	30.5	273.0	0.7
COMPOST SOIL PLUS SAND—NOT STERILIZED																			
11.1	26	19	0	32	68	0	0	15.4	62.6	9.1	36.9	110.0	0.5	20.3	69.2	8.9	30.3	148.0	0.5
19.2	45	12	0	25	58	17	0	16.0	65.0	8.5	34.3	181.0	0.7	15.2	67.4	7.2	32.0	147.0	0.6
31.9	75	43	5	16	53	19	7[2]	32.6	76.7	9.7	22.9	173.0	0.4	29.8	79.4	7.5	20.1	166.0	0.4

[1] In the sterilized soils the presence of rapid-growing colonies of Mucors made it impossible to make accurate counts of the number of fungi. The percentages are therefore based only on the number of bacteria and Actinomyces in the sterilized soils.

[2] A considerable amount of the severe scab in these sets was of the deep or pitted type, while the common type of scab predominated in all other sets.

sterilized soils behaved similarly in this respect and are therefore grouped together in the table. It is clear from the results that when all the tubers were grouped together the low moisture content resulted in the most scab but the individual tubers in these sets became infected only when in contact with moist soil. The author has repeatedly made similar observations in the field. In dry soils scab infections are often found on the under side of the tuber where the soil moisture is slightly higher than that in contact with the upper part.

Soil Moisture Experiment 3.—This experiment was started for the purpose of comparing the effect of the actual moisture content on a dry-weight basis with the percentage of the moisture-holding capacity. A rich composted soil was used which had a moisture-holding capacity of 80.4 per cent as determined by one-centimeter cups. Sand was added to half of this soil at the rate of one part to two parts of soil by volume and the moisture-holding capacity of this mixture was found to be 42.6 per cent. Each of these soils was divided into three sets at 26, 45, and 75 per cent of their respective moisture-holding capacities. A comparison of the first two columns in Table 10 will show that with these percentages the lowest-moisture set with composted soil contained practically the same amount of water on a dry-weight basis as the medium- moisture set in the soil plus sand. Likewise, the medium set in the compost soil was only slightly higher than the high-moisture set in the soil plus sand.

The two soils were again divided into sterilized and unsterilized portions and were inoculated, as in previous experiments, with *A. scabies*. The plants were grown in four-gallon earthenware jars and the moisture requirements were determined by frequent weighings, the water being added both to the surface and to a subsurface reservoir.

Most of the scab was of the common type. The only deep scab occurred in the high-moisture sets of the sterile compost and the unsterilized compost plus sand. The sterilized sets with both soils produced more severe scab than the unsterilized (Table 10). The effect of soil moisture on scab was variable. In the sterilized compost soil and the unsterilized compost plus sand the severity of scab increased greatly with increased soil moistures and the most scab occurred in each instance with the higher percentage of the moisture-holding capacity. This would indicate that the percentage of moisture-holding capacity rather than the actual water present is the more important factor. This conclusion, however, will not hold for the sterilized compost plus sand and the unsterilized compost soil, where there was but little difference in the amount of scab with different soil moistures, or where the medium moisture content was slightly more favorable for scab.

At two periods, approximately 71 days and 95 days after inoculation, soil samples were obtained from each set, each sample being a composite of three borings of the soil for the full depth of the crocks.

The results of the dilution plate counts of bacteria, Actinomyces, and fungi show certain definite trends even though samples were only plated twice during the experiment. It is evident from Table 10 that sterilization

greatly increased the numbers of bacteria, which were still very high 95 days after the start of the experiment. In these sterilized soils there was also a great increase in the numbers of both bacteria and Actinomyces with decreasing moisture contents, the percentage of the moisture-holding capacity being of greater importance than the actual water present. With the higher soil moisture there was also a very marked increase in the percentage of bacteria, with a corresponding decrease in the percentage of Actinomyces. The sharp drop in the percentage of Actinomyces in the medium- and high-moisture sets as compared with the low was particularly pronounced in the sterilized compost plus sand. It was impossible to make accurate counts of the fungi in the sterilized soils because of the abundance of Mucors in the samples, colonies of which rapidly spread over the plates.

In the unsterilized sets there was a much smaller number of bacteria, the highest counts being only about half as much as the lowest counts in the sterilized sets. The numbers of Actinomyces were greater in the unsterilized soils at the medium and high soil moistures and lower with a low soil moisture as compared with the sterilized soils, although the percentages were about the same at the low moisture contents.

There was very little effect of soil moisture on the numbers of microörganisms in the unsterilized soils. The percentages, however, followed the same trend as in the sterilized soils, with an increase in the percentage of bacteria and a decrease in the percentage of Actinomyces with the higher soil moistures. The effect on the percentage of fungi was variable. The effect of soil moistures as regards both numbers and percentages was much less marked in these unsterilized sets as compared with the very marked differences occurring in the sterilized soils.

There was no apparent correlation between either the number or percentage of Actinomyces and the occurrence of scab. In the two sets where the amount of scab increased with increasing moisture contents, there was a corresponding decrease in both numbers and percentages of Actinomyces.

Soil Moisture Experiments 4, 5, and 6.—The influence of the soil moisture content during the period preceding tuber formation, as contrasted with that during the period of tuber development when infection would occur, was studied by experiments in the greenhouse during three successive years.

During the first two years the plants were grown in seven-inch clay pots. In 1930-31 (Experiment 4) each set contained 10 plants, while the following year 20 plants were used for each set. In 1932-33 (Experiment 6) the plants were grown in compartments (78x58 inches) of a greenhouse bench in eight inches of soil, with 24 plants in each compartment.

In all of these tests the soil moisture content was held approximately at the desired point by careful watering so that the low-moisture sets were kept slightly above the wilting point, the medium sets were at about optimum moisture content, and the high sets were kept saturated. While there was naturally considerable variation in soil moisture in an experiment of this type, the three groups covered such a wide range that there was but little danger of overlapping and the plant growth and weight of tubers produced always indicated the great differences. While experiments of this

TABLE 11.—*The effect of soil moisture preceding and during the period of tuber formation upon the development of scab.*

Set No.	Soil moisture: Before tuber set	Soil moisture: After tuber set	Number of tubers	Classes of tubers, determined by percentage of surface scabby: 0	0-2	2-25	25-100
			No.	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
EXPERIMENT 4, 1930-31 (IN CLAY POTS) SOIL INOCULATED AT PLANTING							
1 [1]	Medium	Low	30	13	10	17	60
2 [1]		Medium	34	12	9	30	50
3 [1]		High	35	6	20	51	23
4	Medium	Low	27	15	7	37	41
5		Medium	29	24	28	38	10
6		High	37	46	19	27	8
EXPERIMENT 5, 1931-32 (IN CLAY POTS) SOIL INOCULATED AT PLANTING							
1A	Low	Low	70	3	1	6	90
1B	Medium	Medium	89	1	9	19	71
1C	High	High	107	42	13	22	23
2A	Medium	Low	70	1	3	21	74
2B		Medium	94	2	4	25	69
2C		High	103	9	11	22	58
SOIL INOCULATED AT TUBER SET							
3A	Low	Low	54	28	26	26	20
3B	Medium	Medium	79	65	24	11	0
3C	High	High	101	86	8	6	0
SOIL NOT INOCULATED							
4A	Low	Low	24	54	29	17	0
4B	Medium	Medium	41	76	17	7	0
4C	High	High	47	87	11	2	0
EXPERIMENT 6, 1932-33 (IN BENCHES) SOIL INOCULATED AT PLANTING							
1	Low	Low	41	42	22	24	12
2		Medium	56	39	11	43	7
3		High	107	49	24	26	1
4	Medium	Low	41	29	17	49	5
5		Medium	56	36	11	50	4
6		High	119	29	23	44	4
7	High	Low	69	46	16	32	5
8		Medium	75	21	25	50	3
9		High	121	43	26	26	6
SOIL NOT INOCULATED							
10	Low	Low	32	87	13	0	0
11	Medium	Medium	59	95	2	3	0
12	High	High	136	91	7	2	0

[1] The soil was sterilized before planting. In all other sets the soil was unsterilized.

type are in some ways not as accurate as those in which the water requirements are determined by weighing, they do have the advantage of more uniform distribution of water through the soil. There was also the advantage of a much greater quantity of soil per plant in the bench experiments (Experiment 6, Table 11) and all plants in each set were in one compartment, thus eliminating the individual pot variation occurring in the other tests.

The greatest source of error was in changing the moisture contents at the time of tuber formation. Those in which the moisture content was increased could be changed immediately, but when a lower moisture content was desired it took considerable time for the soil to dry out and the plants required more water to keep them from wilting. As a result the

low moisture contents following previous medium or high moisture were never as low as those started at a low moisture.

The results presented in Table 11 show that the disease was much worse with low than with high moisture contents. This was more evident in the first two years' experiments in clay pots than in the open bench experiments of 1932-33. Scab was also more severe in sterilized than in unsterilized soil (Experiment 4, Table 11) but the effect of soil moisture was similar.

The influence of the soil moisture content preceding the period of tuber formation was evident in Experiment 5, 1931-32. In sets 1B and 2B, which were held at about optimum moisture content throughout the life of the plant, the percentages of scab, particularly of the severe type, were almost identical. Set 2A, in which the moisture content was lowered during tuber development, did not have as much severe scab as 1A, where it had been low during the entire experiment. Likewise 2C produced more scab than 1C, showing the more favorable effect of a medium moisture content as compared with high moisture on the development of the pathogene in the soil preceding the infection period. In the same experiment there was an even greater decrease in scab with constant high soil moistures when the inoculum was not added until the period of tuber development (Set 3), than when the soil was inoculated at the start of the experiment. The uninoculated checks (Set 4) showed the presence of the pathogene in the soil used in this experiment, and the effect of soil moisture was the same as in the inoculated sets.

The inconsistency of soil moisture experiments was evident in Experiment 6, 1932-33, where there was very little effect of soil moisture either preceding tuber formation or during the entire experiment. There were some slight differences in the number of severely infected tubers, but if all infected tubers are considered the differences are either negligible or inconsistent. In this experiment the drying-out process when the moisture content was lowered took longer than in the clay pots and might have influenced the results, but this would not apply to Sets 1, 2, and 3, where the moisture content was low preceding tuber formation. It is not clear why these results were so different from those of the previous year. The amount of inoculum per cubic foot of soil was less than in the previous tests but this should not have affected the influence of soil moisture, which was so evident in the uninoculated soil of Experiment 5.

In Experiment 2 it was noted that stem infection occurred in the inoculated soils that had previously been sterilized and was most severe in the drier soils. This stem infection was again evident in Experiment 5 (Table 12). In contrast with the previous observations, both the number of infected stems and the severity of infection were greatest with the highest soil moisture contents and when the soil had been inoculated at the beginning of the experiment. The least stem infection occurred in the drier, uninoculated soils.

In Experiment 6 soil samples taken at the close of the experiment failed to reveal any significant differences that could be correlated with the occur-

TABLE 12.—*The effect of soil moisture upon the infection of stems with A. scabies (Experiment 5, 1931-32).*[1]

Set No.	Soil moisture		Number of stems	Severity of stem infection			
	Before tuber set	After tuber set		None	Slight	Medium	Severe
			No.	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
			SOIL INOCULATED AT PLANTING				
1A	Low	Low	26	58	27	8	8
1B	Medium	Medium	25	36	32	16	16
1C	High	High	27	22	4	22	52
2A	Medium	Low	32	44	22	22	12
2B		Medium	35	29	29	26	17
2C		High	35	6	6	34	54
			SOIL INOCULATED AT TUBER SET				
3A	Low	Low	30	73	13	13	0
3B	Medium	Medium	34	100	0	0	0
3C	High	High	29	66	17	7	10
			SOIL NOT INOCULATED				
4A	Low	Low	15	100	0	0	0
4B	Medium	Medium	17	100	0	0	0
4C	High	High	10	90	10	0	0

[1] See Table 11, Experiment 5, for data on tuber infection.

rence of scab with regard to either the number or percentages of bacteria, fungi, and Actinomyces as determined by dilution plate cultures.

Three series of samples were taken from each of the low, medium, and high moisture soils of Experiment 6 at monthly intervals, starting three weeks after inoculation. These likewise failed to reveal any significant differences that could be correlated with the occurrence of scab. There was an increase in the percentage of Actinomyces with increasing moisture contents in both the inoculated and uninoculated soils (Table 13). This result is just the opposite of what might be expected on the basis of the published data of Dippenaar (4) and other workers.

In addition to the usual soil samples for the dilution plates, a set of samples was obtained from the soil in close contact with the potato tubers. The tubers were carefully removed from the soil, all the loosely adhering

TABLE 13.—*The effect of soil moisture upon the numbers of bacteria, Actinomyces, and fungi in inoculated and uninoculated soil.*[1]

Per cent soil moisture (dry weight)	Total number of organisms	Percentage of organisms in each group		
		Bacteria	Actinomyces	Fungi
Per cent	*Millions*	*Per cent*	*Per cent*	*Per cent*
	INOCULATED SOIL			
Low 16.6	12.1	79.9	18.8	1.3
Medium 21.1	9.5	79.2	19.3	1.6
High 28.7	10.4	76.4	21.9	1.7
	UNINOCULATED SOIL			
Low 16.1	11.4	82.7	15.9	1.3
Medium 23.6	13.1	81.6	17.4	1.1
High 30.3	13.4	78.7	19.7	1.5

[1] The data presented are the averages of three series of samples taken at intervals of one month, the first sample being taken three weeks after inoculation. All data are on the dry-weight basis. For data on scab see Table 11, Experiment 6, 1932-33.

soil was detached by shaking, and the tubers were then immersed in a measured amount of sterile water and the soil removed by vigorous shaking. Estimated dilutions were made from this suspension after the tuber was removed and plates were then poured. The exact dilution was calculated later by evaporating the unused portion of the suspension to dryness and determining the dry weight of soil in the original suspension. This method has been used repeatedly by the author in field studies with roots and underground plant parts and always reveals a much higher number of microorganisms than do the usual soil samples. In the present test the total number of bacteria and Actinomyces in the low-moisture series was 294 million, with only 0.7 per cent Actinomyces. In the medium-moisture set it was 90 million, with 0.8 per cent Actinomyces, while in the high-moisture set it was 102 million and 1.9 per cent. The total count was 10 to 20 times as great as in the usual soil sample (Table 13), because of the great increase in bacteria rather than Actinomyces, which were no more numerous and the percentages of which were of course greatly reduced. The percentage of Actinomyces, however, was increased with an increase in the soil moisture content.

The results obtained by direct microscopic examinations of slides buried for two weeks in the soils likewise failed to aid in the interpretation of the experimental data previously presented. The data given in Table 14 do not check with the results of the dilution plates. Actinomyces in the form of both hyphae and spores were much more numerous in the dry soils. This corresponds to the results obtained by Dippenaar (4).

In the preceding experiments there was some indication of an effect of the soil moisture content during the period preceding tuber formation upon

TABLE 14.—*Effect of soil moisture upon the development of Actinomyces, bacteria, and fungi in soil inoculated with A. scabies.*

Soil moisture		Actinomyces			Bacteria	Fungi
Previous	During slide exposure	Vegetative hyphae	Spirals and sporogenous hyphae	Free spores		
SLIDES PLACED IN SOIL TEN WEEKS AFTER INOCULATION [1]						
Low	Low	++++ [2]	+++	+++	++	+
Medium	Medium	+++	++++	++	++	++
High	High	+	++	+	+++	++
AVERAGE OF THREE SETS OF SLIDES PLACED IN THE SOIL 14, 16, AND 18 WEEKS AFTER INOCULATION—SOIL MOISTURES CHANGED AFTER 14 WEEKS						
Low	Low	+++	+++	+++	++	+
Low	Medium	+	++	++	+	+
Low	High	++	+	+	++	++
Medium	Low	++	++	++	++	+
Medium	Medium	++	+	+	+++	+
Medium	High	+	+	+	++	+
High	Low	+++	+++	++	+	+
High	Medium	+++	++	+++	++	+
High	High	+	+	—	++	++

[1] Duplicate slides were used and aproximately 200 fields were examined on each slide.
[2] Symbols are: —, negative; +, trace; ++, few; +++, common; ++++, abundant.

the incidence of scab. It was thought possible that if this period was extended over some months before planting, the differences resulting from the various treatments might be more clear cut. Accordingly, Experiment 7 was planned, which was essentially a continuation of Experiment 6 (1932-33, Table 11). No additional inoculum was added to the soil during the 1933-34 tests. Immediately after harvest of the tubers of Experiment 6 in April, 1933, all the soils were brought to optimum moisture content and held at that point for about two months. On June 15 the new treatments were started and these were not changed until November 25. One week later all sets were planted with healthy seed pieces previously sprouted in sterile sand. The complete history of the soils for the two-year period and the amount of scab in 1934 are shown in Table 15.

TABLE 15.—*The effect of soil moisture and other treatments before planting upon development of scab and upon the soil microflora (Soil Moisture Experiment 7.)*

Item	Set Number [1]								
	1	2	3	4	5	6	7	8	9
Moisture content and history of the soil (L=low, M=Medium, H=High)									
Planting, Nov. 15, 1932 to tuber set [2]	L	L	L	M	M	M	H	H	H
Tuber set to harvest, Apr. 8, 1933	L	M	H	H	M	L	H	M	L
April 8 to June 15, 1933	M	M	M	M	M	M	M	M	M
June 15 to planting, Nov. 30, 1933	Air dry	Air dry	H	H	M	Sand [3]	Manure [3]	Barley [3]	Naphthalene [3]
After planting, 1933-34 [4]	L	M	M	H	M	M	M	M	M
Number of tubers	25	34	85	109	63	41	60	61	41
Weight of tubers (g.)	1,250	3,000	8,600	10,600	3,600	2,450	3,800	3,450	2,550
Classes of tubers determined by percentage of area scabby:									
0 (*p.ct.*)	100.0	100.0	4.7	52.3	92.1	95.1	95.0	82.0	85.4
0-2 (*p.ct.*)	0.0	0.0	36.5	38.5	7.9	2.4	3.3	18.0	12.2
2-25 (*p.ct.*)	0.0	0.0	55.3	8.3	0.0	2.4	1.7	0.0	2.4
25-100 (*p.ct.*)	0.0	0.0	3.5	0.9	0.0	0.0	0.0	0.0	0.0
Microörganisms per gram of soil (dry weight)									
Total (*millions*)	6.2	4.6	10.2	13.2	3.6	1.9	5.8	4.2	4.9
Actinomyces (*millions*)	3.2	1.2	2.4	1.8	0.6	0.5	0.9	1.1	1.2
Actinomyces (*p.ct.*)	51.6	26.2	23.1	13.6	17.9	26.6	15.8	26.7	23.8
Bacteria (*p.ct.*)	45.1	69.6	76.0	85.6	79.4	70.0	82.9	71.0	72.6
Fungi (*p.ct.*)	3.2	4.3	0.9	0.7	2.8	3.4	1.4	2.3	3.6

[1] Each set consisted of 25 plants.

[2] All soils were held at a medium moisture content for one month after inoculation October 15, 1932.

[3] Sets 6 to 9 were held at medium moisture content during this period. Sand and well-rotted stable manure were added at the rate of 1 part to 2 of soil by volume. The barley was grown during the summer and turned under about a month before planting. Naphthalene was added at the rate of 1 pound to 21 cubic feet of soil.

[4] At the time of sampling for dilution plates the soil moisture in Set 1 was at 53 per cent of moisture-holding capacity; the medium-moisture Sets 2, 3, 5, and 7 varied from 67 to 73 per cent; and the high-moisture Set 4 was 90 per cent of the moisture-holding capacity. Sets 6, 8, and 9 varied from low to medium moisture.

The plants in Set 4 with high moisture grew the most rapidly and produced the largest crop; those in Set 3 where the moisture content was dropped from high to medium at planting time gave nearly as large a yield. This was partly due to the fact that during the first few weeks of

the experiment this soil was still above the moisture content of the other sets that had been previously kept at a medium moisture content. Set 1, with a minimum moisture content, produced the smallest crop. Manure as applied in Set 7 gave only a slightly increased yield over the untreated Set 5.

The results as regards scab infection were very clear cut but did not check with many of the previous experiments. A large amount of scab occurred only in Sets 3 and 4. These were the same two sets that had the largest crop. The scab in these two sets was also of a more severe type with some deep scab, while the other sets were mostly common scab. The dry soil of Set 1 and the medium moisture soil of Set 2 which had previously been air dry were entirely free from scab. There was a great difference in both scab and tuber development in Set 2, previously dry, and Set 3, previously at high moisture content, although both sets were held at a medium moisture content during tuber formation. The addition of manure in Set 7 resulted in slightly less scab than in the untreated Set 5, although the difference was hardly great enough to be significant. Neither the turning under of barley nor the addition of naphthalene reduced scab; in fact there was slightly more in these sets than in any of the others except Sets 3 and 4.

Soil samples were plated out from all sets during the period of tuber formation, but neither the number nor the percentage of Actinomyces could be correlated with the amount of scab (Table 15). The highest number and percentage (51.6 per cent) of Actinomyces occurred in the dry soil of Set 1, which had no scab. The lowest percentage (13.6) of Actinomyces occurred in the high-moisture soil of Set 4, which had next to the largest amount of scab. Set 3, with the largest amount of scab, had next to the highest number of Actinomyces and a percentage about the same as the other sets that had very little scab. The addition of manure to Set 7 did not increase the number or percentage of Actinomyces. Neither the turning under of barley nor the addition of naphthalene or sand had any great effect upon the percentage of Actinomyces, all of which were slightly higher than the untreated soil in Set 5, although there was a marked reduction in the total number of organisms with the addition of sand.

Slides were placed in the soils of Sets 1, 3, 4, and 5 about five weeks after planting and left for ten days. An examination of these showed abundant hyphae of Actinomyces in Sets 3 and 5, with only a slight amount in the low-moisture Set 1 and the high-moisture Set. 4. Spirals were present only in Set 5, while free spores were abundant in Sets 1 and 5 with only a few present in Sets 3 and 4.

The results obtained in this experiment, which was a continuation of the previous year's test without the addition of new inoculum, might have been greatly influenced by the preceding soil treatments. It is possible that the air-drying of the soil in Sets 1 and 2, where the soil moisture was as low as 3.7 per cent for four months before planting, resulted in partial sterilization which greatly reduced the numbers of the scab organism, although the number and percentage of all Actinomyces were very high in Set 1 (Table 15). Certainly the data do not indicate that *A. scabies* is favored by such dry conditions or that it is inhibited by high soil moistures such as occurred

in Sets 3 and 4 before planting and in Set 4 during the growth of the plants. These results are very different from those reported by Dippenaar (4) and Sanford (16, 17). Likewise, the data for the manured soil in Set 7 do not bear out the oft-repeated assertion that the application of manure increases scab.

In order to eliminate the possibility of any variation in the amounts of inoculum at the start of the test, such as occurred in Experiment 7, another test (Experiment 8) was started in the winter of 1934-35. This experiment was carried out in the same benches as the preceding experiment but a new mixture of unsterilized, composted soil was placed in the compartments. The soil in each of the nine compartments was inoculated June 1 with equal portions of a suspension of *A. scabies* N-23, made up at the rate of 50 petri-dish cultures per compartment. The inoculum was thoroughly mixed with the soils, which were then held at about an optimum moisture content until July 10. At this time they were divided into low, medium, and high moisture groups of three compartments each. The low-moisture group was allowed to become air dry. The medium group was held at about the same moisture as during the preceding 40 days and the high-moisture group was kept saturated by frequent watering. They were held at these moisture contents until October 13, when each of the above three groups was divided into a low, medium, and high moisture set as shown in Table 16. It took one month to dry out the high-moisture soils to the desired point and 24 seed potatoes were then planted in each compartment (November 13), after having been sprouted in sterilized sand. The experiment was completed and the tubers examined February 15.

The moisture-holding capacity of the soil was 42.0 per cent. The soil moisture content was determined by composite samples taken weekly and the averages are shown in Table 16 for each set. While there was considerable variation from week to week, the extremes did not overlap and the soils at the end of the experiment were found to be more uniform in moisture content than in some of the previous experiments, where water was applied after weighing individual containers. The average for the three low moisture sets was 43.0 per cent of the moisture-holding capacity; for the medium, 56.4 per cent; and for the high, 82.7 per cent.

During the summer months the daily mean soil temperature in the soil of the medium-moisture compartments averaged 82.5°, the maximum temperature recorded being 99° and the minimum 67° F. During the winter the greenhouse was held at an air temperature of about 72°.

The results of the experiment are presented in Tables 16 and 17. Both the number and weight of tubers produced were directly correlated with the moisture content of the soil. The greatest yield was from the high-moisture set previously held at a low moisture content. Probably the reason for the higher yield in Set 7 than in the other low-moisture sets was the fact that it had previously been at a high moisture content and was always slightly above the moisture contents of the other low series.

It is evident from the data in Table 16 that high soil moisture previous to time of planting increased the amount of scab in Sets 7 and 8, as com-

TABLE 16.—*The influence of soil moisture preceding and during the growth of the plant upon the development of potato scab (Soil Moisture Experiment 8).*

Set No.[1]	Moisture content [2] (approximate and per cent dry weight)				Number of tubers	Weight of tubers	Classes of tubers determined by percentage of surface scabby							
	Before planting		After planting				On number basis				On weight basis			
							0	0-2	2-25	25-100	0	0-2	2-25	25-100
	P.ct			*P.ct.*	*No.*	*Grams*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
1	Air dry		Low	17.5	20	385	60.0	40.0	0	0	53.2	46.8	0	0
2	Air dry	3.3	Medium	21.1	69	1,670	95.7	4.3	0	0	91.0	9.0	0	0
3	Air dry		High	31.5	126	6,060	93.6	6.4	0	0	91.4	8.6	0	0
4	Medium		Low	17.0	25	470	60.0	40.0	0	0	51.1	48.9	0	0
5	Medium	24.2	Medium	25.0	47	1,250	95.8	4.2	0	0	96.0	4.0	0	0
6	Medium		High	34.1	85	5,360	82.4	17.6	0	0	75.4	24.6	0	0
7	High		Low	19.5	53	1,310	41.5	52.8	3.8	2.0	20.6	71.8	5.3	2.3
8	High	42.1	Medium	24.6	81	3,440	53.1	40.8	4.9	1.2	55.8	39.0	4.6	0.6
9	High		High	38.4	107	5,630	91.6	6.5	0.9	0.9	90.6	7.5	0.5	1.4

[1] Each set consisted of 24 plants.

[2] Soil was inoculated June 1 and held at a medium moisture content until July 10 and then changed and held at the desired moisture contents until October 13, when they were changed to the moisture contents desired after planting. Experiment planted November 13.

pared with the comparable sets held at the same moisture contents during growth of the plant. This was not true of Set 9, which was held at a high moisture content throughout the experiment.

It can also be concluded from the data that low soil moistures during the growth of the plant (Sets 1, 4, and 7) favored the development of scab. There is no apparent explanation for the greater percentage of scab in Set 6 at a high moisture content than in the medium-moisture Set 5 of the same series.

The counts of Actinomyces, bacteria, and fungi were made by the dilution plate method three times during the experiment, the first about one month after inoculation, the second just before the soil moistures were changed (October 13) and the last at the end of the experiment. The results are presented in Table 17.

The percentage of Actinomyces one month after inoculation and after one month at a medium moisture content averaged about 28 per cent and was fairly uniform in the different compartments. The second sampling (October 13), made about three months after the soil moistures had been changed, revealed some marked differences in the three groups. There was a great decrease in the total number of organisms in the low-moisture sets (1, 2, and 3) due to the fact that these soils were allowed to become air dry. The effect of desiccation was greatest on the bacterial flora, while the percentages of Actinomyces and fungi were greater than at any other time during the experiment. The higher soil moisture in the medium sets (4, 5, and 6) resulted in a greater total number of organisms and a lower percentage of Actinomyces and fungi. Raising the moisture content still higher failed to have any further depressing effect on the percentage of Actinomyces, although the total number of organisms doubled. The samples taken at the close of the experiment (February 15) showed a considerable increase in the total numbers of organisms at all moisture contents, probably due to the effect of the root system of the potato plants. Unfortunately the data for Sets 1 and 4, in which the greatest amount of scab occurred in their relative series, are not available. There was an increase in the numbers of both Actinomyces and bacteria in Sets 7, 8, and 9, which had previously been held at high moisture contents. It is significant that these were the same three sets in which the greatest amount of scab occurred in comparison with the respective moisture contents in the other series. It is likewise noticeable that the two sets having the lowest number of Actinomyces, Sets 2 and 5, likewise had the least scab and Set 6 with a greater number had a larger amount of scab. It is possible that if data were available for the two low-moisture sets (1 and 3) a more refinite correlation could be made.

An examination of the soil microflora was attempted by the Cholodny technique at intervals throughout the experiment. Slides placed in the soils during the first month after inoculation and left for two weeks showed an abundance of Actinomyces on all slides. Long strands of mycelium, as well as many mycelial fragments, chains, free spores, and a few spirals were observed. Slides buried for two weeks during the period before plant-

TABLE 17.—*Influence of soil moisture upon Actinomyces, bacteria, and fungi in unsterilized, composted soil inoculated with A. scabies (Experiment 8, 1934-35).*[1]

Set No.	Soil moisture content		Soil moisture at sampling	Microörganisms per gram of soil (dry weight)			
	Before planting	After planting Nov. 13		Total organisms	Actinomyces	Bacteria	Fungi
			Per cent	*Millions*	*Thousands*	*Per cent*	*Per cent*	*Per cent*
				SAMPLED JULY 3, MEDIUM MOISTURE[2]			
1, 2, 3	—	—	25.6	3.4	839	24.4	72.4	3.2
4, 5, 6	—	—	28.4	3.6	928	26.1	70.8	3.1
7, 8, 9	—	—	29.5	3.9	891	23.1	73.8	3.1
				SAMPLED OCTOBER 13				
1, 2, 3	Air dry	—	3.3	0.7	226	34.0	56.8	9.2
4, 5, 6	Medium	—	24.2	2.3	574	24.6	71.2	4.2
7, 8, 9	High	—	42.1	4.8	1,151	24.2	73.5	2.3
				SAMPLED FEBRUARY 15				
2	Air dry	Medium	21.9	10.2	727	7.1	91.7	1.1
3	Air dry	High	33.0	10.8	1,123	10.3	88.7	0.9
5	Medium	Medium	23.4	9.9	940	9.5	89.4	1.1
6	Medium	High	33.8	9.8	1,112	11.4	87.4	1.2
7	High	Low	21.0	13.7	1,246	9.1	90.1	0.7
8	High	Medium	26.4	14.4	1,260	8.7	90.6	0.7
9	High	High	38.8	13.6	1,131	8.3	90.9	0.8

[1] See Table 16 for scab data on the same soils.
[2] These plots had been held at a medium moisture content for 33 days before sampling.

TABLE 18.—*The effect of soil aeration upon the development of scab.*

Set No.	Soil treatment		Number of tubers	Weight of tubers	Classes of tubers, determined by percentage of surface scabby									
	Before tuber formation	During tuber formation			On number basis					On weight basis				
					0	0-2	2-25	25-75	75-100	0	0-2	2-25	25-75	75-100
			No.	*Grams*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*
1	Open	Open	20	650	0	0	20	70	10	0	0	27	55	18
2	Sealed	Sealed	16	460	6	31	25	38	0	9	21	33	38	0
3	Sealed	Open	13	625	31	8	54	8	0	18	14	66	2	0
4	Open	Sealed	22	670	0	0	45	41	14	0	0	28	54	18

ing, about three months after the soil moitures were changed, showed a great decrease in the numbers of Actinomyces in the soils held at low moistures, but this may have been due to the fact that this technique is not very satisfactory with dry soils owing to the poor contact between the slide and the soil. The greatest number of Actinomyces were found in the soils held at a medium moisture content, and only traces were found in the soils at the high moisture content. The slides buried about two months after planting (two months after the second change in the soil moisture content) failed to reveal any significant differences and there was no correlation between either the numbers or the type of growth of Actinomyces as revealed on these slides and the numbers of Actinomyces as determined by the dilution plate method or the amount of scab on potato tubers.

The only conclusion to be drawn from this experiment is that while low soil moistures during the growing season favored the production of scab, high moistures preceding planting likewise increased scab in this experiment, provided favorable conditions for infection existed during the period of tuber development.

THE EFFECT OF SOIL AERATION UPON POTATO SCAB

Sanford (17) found that *A. scabies* in pure culture required an abundant supply of oxygen for all phases of its life cycle and he therefore suggested that lack of sufficient oxygen might be a limiting factor in the soil and "help to explain why scab is not so prevalent in wet soils as in drier ones". On the basis of observational evidence in western Nebraska, this theory was inadequate to explain the common occurrence of severe attacks of scab following heavy packing rains and in the low portions of irrigated fields, particularly when the fields could not be subsequently cultivated. A few simple tests were therefore planned to obtain some experimental evidence on the effect of lack of aeration of the soil upon the development of scab.

Since these tests were made, Dippenaar (4) has published the results of his experiments, in which he tested the effect of different degrees of forced aeration in soils of different moisture contents. His results did not show any increased amount of scab in the aerated as compared with the unaerated controls.

In the following tests instead of forcing air through the soil it was considered that normal cultivation of the surface soil was sufficient for the development of severe scab and that a decrease in aeration would test the possibility of insufficient oxygen acting as a limiting factor. It was also considered that the effect of aeration on the infection phenomena might be different from the effect upon the growth of the organism in the soil preceding tuber formation. An attempt was made to simulate the conditions occurring in the field after a heavy packing rain and subsequent hardening of the surface soil by covering the soil with an inch layer of closely packed, wet clay in contrast with a frequent cultivation of the surface soil.

The plants were grown in an unsterilized mixture of composted soil and sand in four-gallon glazed earthenware jars. The soil was inoculated

one week after planting with a suspension of *A. scabies* N-23. One series of 10 plants was covered with clay as soon as the sprouts emerged and left that way until maturity. Another series was covered the same way but the clay was removed at the time the tubers started to set and cultivated thereafter. A third and fourth series of 10 plants each were treated conversely to the first two, one being cultivated throughout the experiment and the other cultivated until time of tuber set and then covered with clay.

From 90 to 100 per cent of the tubers in each series were scabby, most of the scab being of the common type with little pit scab present. The amount of infection was slightly greater in the series that was cultivated throughout the experiment, but there were no significant differences between the other three series.

It was noted in this experiment that the tubers on the plants that were covered with clay during tuber formation had all formed at the surface of the soil with the upper side in contact with the clay. In every instance the scab lesions on such tubers occurred on the lower side in contact with the soil, while the upper portion was always healthy.

The above experiment was repeated with a similar number of plants grown and inoculated in the same way except that the soil was sealed by pouring over it a one-half-inch layer of paraffin as soon as the sprouts had emerged, and the soil was kept at an optimum moisture content by weighing and adding water through a tube to a three-inch clay pot reservoir in the center of the soil mass. The results of the experiment are given in Table 18.

It is evident from the data that a lack of aeration inhibited the development of scab. It is also obvious from a comparison of Sets 3 and 4 that a lack of aeration was of much greater importance during the period preceding tuber formation than later in the growth of the plants. Apparently the effect of the treatment was upon the organism rather than upon the host and a lack of aeration did not affect the vegetative development of the organism. It is possible of course that some small tubers had formed and become infected in Set 4 before the soil was sealed. However, developing tubers are susceptible during their entire growth period, so that the comparatively low percentage of infection in Set 3 must be explained on the basis of lack of inoculum due to the preceding lack of aeration.

Further experiments on this point combined with a study of the organism in the soil by dilution plates and slides are needed for an exact understanding of the effect of aeration on the organism in the soil.

DISCUSSION

It has long been considered that environmental factors, particularly those of the soil environment, are of major importance in determining the severity of potato scab. The literature of the subject is replete with observations of the effects of these factors, although accurate experimental work is not so abundant. Many contradictory statements have resulted from the observational evidence and while most of the experimental evidence is in fair agreement, it is often difficult, if not impossible, to correlate the

occurrence of the disease in the field with published experimental findings obtained under controlled conditions. Temperature, moisture, and to a less extent aeration fluctuate greatly during the growing season in comparison with the other more stable factors, such as reaction, soil type, and organic matter. It is quite probable that these fluctuating factors are the cause of the many contradictory observations and for this reason they were selected for investigation in the present study.

It is obvious that these factors may exert their influence in two ways, either by their effect upon the pathogene and its host at the time of infection or by their effect upon *A. scabies* in the soil previous to infection. This latter phase of the problem has received very little attention in previous investigations, and the experiments in the present paper were therefore designed in an attempt to throw some light on this question.

Effect of competing organisms.—It has been shown (Table 1) that the severity of scab is directly dependent upon the amount of inoculum in the soil. It can be concluded that the amount of inoculum will be conditioned by the direct action of the environmental factors or indirectly by their influence on the soil microflora, which may be either beneficial or antagonistic to the pathogene.

The increased infection resulting from soil sterilization and subsequent inoculation is apparently due to the lack of competition of soil microorganisms rather than to changes in the physical or chemical structure of the soil. The addition to such soils of a filtrate from unsterilized soil tends to counteract this effect, and the effect of sterilization is greatly reduced if the inoculum is not added until after saprophytic organisms have become established in the soil. Tests of the antagonistic effect of *A. praecox* as reported by Millard and Taylor (15) failed to substantiate their report of its potency in reducing the amount of scab. No evidence was obtained as to whether the effect of the soil microflora upon *A. scabies* was due to specific organisms, related groups of organisms, or simply to numbers of competing organisms.

Soil temperature.—If the soil microflora exerts such a pronounced effect upon *A. scabies* as was shown in the soil sterilization experiments, it would be expected that any factors influencing the development of the soil microflora would have a profound effect upon the development of *A. scabies* and therefore upon the scab-producing power of the soil. This was demonstrated by the soil temperature studies in which the soil was subjected to the desired temperatures for 70 to 90 days previous to tuber formation, after which they were all transferred to a uniform temperature of 22° C. determined by Jones, McKinney, and Fellows (9) and Dippenaar (4) to be a favorable temperature for infection. With sterilized soil the most scab occurred when the soils had been incubated at 22° and 26° C., with practically as much scab at 30°. With soils incubated at lower temperatures the amount of scab was less, reaching a minimum in the soil held at 6°. Contrasted with this, there was no consistent effect of temperature in the unsterilized soils. It is possible that additional experiments involving larger numbers or the incubation of the organism in the soil for longer periods of time might

show a greater effect of temperature on unsterilized soils than was evident in this single experiment. It is evident, however, that in experiments where sterilized soil is inoculated and held at constant temperatures from planting to maturity, as in the work of Jones, McKinney, and Fellows (9), the results are due to the effect of temperature not only upon infection but also upon the growth of the organism in the soil, and therefore the differences in scab obtained at different temperatures are magnified.

Soil moisture.—High soil moistures have been claimed by various workres (4, 12, 16) to reduce scab infection, while a few papers record the opposite effect. Records of fields in western Nebraska show that scab is usually more severe in seasons of high rainfall than in the dry years. Because of the deficiency of moisture, scab is never a problem in years of low yields.

The experiments reported in this paper fail to reveal clearly the effect of soil moisture upon the occurrence of scab as shown by Dippenaar (4). The soil moisture in his experiments was regulated by periodic weighings, but in the few experiments where this was done in the present investigation the results were more variable than where the soil moisture was held approximately at the minimum optimum, and maximum for growth. This method allowed for larger numbers of plants and the plants produced a greater number of larger tubers. The method of recording scab was also different. These differences in methods, however, can hardly account for the great variation in the results. In general, the results agreed with Dippenaar as to the greater amount of scab occurring in soils that were dry during the infection period; however, in Soil Moisture Experiment 3 (Table 10) there was clearly an increase in scab with increased moisture in two of the four sets. In Experiment 6 (Table 11) there was no effect of soil moisture, whereas in Experiments 4 and 5 more scab occurred in the dry soil. In Experiment 7 (Table 15) the only severe scab occurred at medium and high moisture contents, while in Experiment 8 (Table 16) more scab occurred in the dry soil.

It is quite possible that the reason for these variable results lies in the lack of proper experimental technique. Even when the water to be added is determined by periodic weighings it is self-evident that the soil moisture is at the desired point only immediately after watering. It is also true that in such experiments we are dealing with the average soil moisture in the containers, whereas the determining factor is the moisture present at the point of infection. This is best exemplified by the data presented in Table 9 The greatest amount of scab occurred in the dry soils in which the soil moisture was unevenly distributed, and the scab occurred chiefly upon the portions of the tubers that were in contact with moist soil.

In general the scab occurring in the high moisture soils was severe and often of the pitted type, in contrast to Dippenaar's observations of high soil moisture allowing the tuber to slough off the scab infection and heal over the pustule. There was no evidence of this occurring in these experiments, nor was there any effective control of scab by high moistures even when it was more severe in the drier soils.

The data in the one experiment conducted to determine the effect of soil moisture in relation to soil type and moisture-holding capacity were too insufficient and variable to permit definite conclusions. Experiments dealing with soil types are bound to be influenced greatly by differences in organic matter and aeration, which also affect the soil microflora. The limited evidence obtained indicates that the proportion of soil moisture to the moisture-holding capacity is probably of greater significance than the actual percentage of water on a dry-weight basis, and that caution should be used in comparing the effects of soil-moisture contents of different types of soil.

As previously stated, there is very little published information dealing with the effect of soil moisture preceding infection—that is, upon the development of the inoculum. Working with pure cultures of *A. scabies* in sterilized soil in flasks Sanford (17) came to the conclusion that "the inoculum of *A. scabies* appeared to increase best at a soil moisture content about optimum for plant growth". Dippenaar (4), employing the Cholodny slide technique, came to the conclusion that high soil moisture favored the development of bacteria which were antagonistic to the scab organism and that spore germination and growth of *A. scabies* were inhibited under these conditions. The Actinomyces in general are usually said to be favored by low soil moistures but Lutman, Livingston, and Schmidt (11) found the greatest number in field soils in winter with high soil moistures.

In the present investigation the largest amounts of scab occurred in the soils that had been held at medium or high moisture contents for some months previous to planting. While the results were not consistent in repeated tests (Experiments 6, 7, and 8) there was no evidence that high soil moistures (saturation in these experiments) have any depressing effect on the scab organisms. On the contrary, in one experiment air-drying of the soil previous to planting greatly decreased the amount of scab.

Plate counts and soil slides did not reveal any consistent relation between Actinomyces and scab, and there was considerable variation in the effect of moisture on the numbers and percentages of Actinomyces. In general the percentage of Actinomyces was greatest in the dry soils. In two experiments (Tables 10 and 15) both the number and percentage were highest in dry soils, while in another experiment (Table 13) there was a slight increase in the percentage with high moisture and in Experiment 8 (Table 17) there was a greater number and percentage of Actinomyces in the high as compared with medium moisture, except for those soils originally at a high moisture.

The soil slides did not check with the results of dilution plates. Actinomyces were more abundant on the slides in the dry soils but they were not completely eliminated by high moisture, as in the experiments reported by Dippenaar. The fact that in these experiments the slides were buried in soil containing roots of living plants, while Dippenaar conducted his tests in soil without plants, may account for the difference.

Lutman and co-workers concluded that Actinomyces occur in the soil in the form of mycelial fragments rather than as spores. Dippenaar

considered that a low moisture content favored not only spore germination and vegetative development but also sporulation. Sanford (17) suspected that the lack of spore germination at high humidities may have been due to a lack of oxygen resulting from the film of water around the spores.

While great stress is often laid upon the numbers of Actinomyces as determined by dilution plate counts in relation to soil moisture and other factors, it should be borne in mind that the causal organism must be growing vegetatively if it is to cause infection. High plate counts often indicate sporulation or fragmentation of mycelium. Conversely, low counts may indicate vegetative development and this may in turn be correlated with high infection. Of course it is possible that germination, growth and sporulation all occur at their maximum under similar conditions, as indicated by Dippenaar, although this is not a common biological phenomenon. At least it is reasonable to assume that conditions favorable for sporulation would increase the scab-producing power of the soil and that if this were followed by conditions favorable for vegetative development, more infection would result. This phase of the problem needs more investigation before a comprehensive knowledge can be obtained of the development of the causal organism in the soil in relation to the production of the disease.

Soil aeration.—Soil aeration was found to be essential for the development of the disease, as previously indicated by other investigators, but it was also found that a lack of aeration during the period preceding tuber formation had a greater influence upon the disease than during the infection period. This was probably due to a lack of development of the parasite in the soil when insufficient oxygen was present.

Type of scab.—In the experiments reported in this paper there was no evidence of a correlation between different types of scab and the strain or species of Actinomyces, with the possible exception of superficial scab of the type shown in Plate 1, which occurred only in the unsterilized soils. Common, raised, and deep scab similar to the types described by Millard and Burr often occurred on the same tuber grown in sterilized soil inoculated with a single pure culture of *A. scabies*. Additional tests have indicated that the type of scab is more closely correlated with the host reaction, the environment, and the virulence of the culture than with the identity of the species or strain.

The difficulty of obtaining uniform results and in interpreting such data as those presented in this and other papers dealing with environmental factors, is largely due to the fact that our experimental technique is inadequate for a proper investigation of the problem. While we can measure gross differences in the soil mass in which the plants are growing, the conditions existing in the microenvironment immediately surrounding the roots and tubers is not measurable by present methods and it is these undetermined conditions that undoubtedly have the greatest influence upon the development of the organism.

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